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Analysis of Honey*

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Carbohydrates in Honey

Since the last report, a limited amount of additional collaborative testing has been done on the selective adsorption method for honey sugars. The method has been accepted as first action by the Association (1).[1]

The collaborator analyzed three of the four samples studied in the 1958 report (2). They had been kept in deep freeze since that time to minimize changes. Each sample was run in duplicate using two columns. The results are given in Table 1 and also in Table 1 are shown the analyses of the same samples by the 1958 collaborators (2). The 1959 collaborator found it necessary to calibrate the procedure rather than to use the equations given in the method for fructose and dextrose; use of the latter gave results for known sugar mixtures 2–5% (of the sugar present) high. A statement regarding this necessity has been added to the method. The collaborator experienced difficulty in obtaining a sample of Darco G-60 of sufficiently fast flow rate for use. Flow rate of the charcoal can be somewhat improved by removing "fines" by screening.

Table 2 shows a comparison of the interlaboratory average values (Laboratory 1, 1958, one adsorption by each of three analysts; Laboratory 2, 1959, 2 adsorptions by one analyst). It is felt that agreement between laboratories in this limited study is excellent.

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[1] *This Journal*, **42**, 50, 344 (1959), first line under "Higher Sugars as 'Dextrin'" should read "Pipet 25 ml . . ."

Table 1. Collaborative analyses of three honey samples by selective adsorption method[a]

Sample	Analyst	Dextrose (%)	Fructose (%)	Sucrose (%)	Maltose (%)	Higher Sugars (%)
222	1958–1	27.27	36.40	0.58	10.58	2.02
	1958–2	26.47	37.20	0.94	10.02	2.20
	1958–3	27.69	37.36	0.73	10.79	2.23
	1959–1	26.40	37.06	0.84	10.16	2.01
	1959–1	26.81	36.90	0.64	10.18	1.71
260	1958–1	33.00	41.00	1.52	7.02	0.82
	1958–2	32.44	40.61	1.48	6.98	1.00
	1958–3	33.04	40.92	1.59	7.30	0.78
	1959–1	32.76	40.54	1.73	7.08	0.66
	1959–1	32.77	40.36	1.61	7.12	0.68
264	1958–1	28.62	38.19	0.48	7.99	0.89
	1958–2	28.38	38.68	0.80	7.46	0.96
	1958–3	28.40	38.50	0.79	7.76	0.88
	1959–1	28.41	38.30	0.50	7.96	0.98
	1959–1	28.31	38.48	0.49	7.85	0.84
Std.	1958	0.38	0.42	0.14	0.28	0.08
deviation	1958–9	0.34	0.25	0.12	0.22	0.14

[a] The first three values in each group were previously published (2).

Table 2. Comparison of interlaboratory average sugar analyses of three honey samples

Sample	Laboratory	Dextrose (%)	Fructose (%)	Sucrose (%)	Maltose (%)	Higher Sugars (%)
222	1	27.14	36.99	0.75	10.46	2.15
	2	26.61	36.98	0.74	10.17	1.86
	Difference	0.53	0.01	0.01	0.29	0.29
260	1	32.83	40.84	1.53	7.10	0.87
	2	32.77	40.45	1.67	7.10	0.67
	Difference	0.06	0.39	0.14	0.00	0.20
264	1	28.47	38.46	0.69	7.74	0.91
	2	28.36	38.39	0.50	7.91	0.91
	Difference	0.11	0.07	0.19	0.17	0.00
Interlaboratory differences within:						
1 S.D.		2	2	1	2	1
2 S.D.		1	1	2	1	1
3 S.D.		0	0	0	0	1

Table 3. Detection of commercial glucose (corn sirup) in honey. Comparison of methods

Sample No.	Corn Sirup Content (%)	Coll. 1		Coll. 2		Coll. 3		Coll. 4		Coll. 5	
		New	Old[a]	New	Old[a]	New	Old[a]	New	Old[a]	New	Old[a]
A1	20	+ (strong)	+	+	−	+	−	+ (strong)	inconclusive (see text)	+ (strongest)	−
A2	0	−	−	−	−	−	−	−		− (possible trace)	−
A3	10	+	−	+	−	+ (less than A1)	−	+		+	−
A4	10	+ (weak)	−	+	−	+ (trace)	−	+		+	−
A5	0	−	−	−	−	−	−	−		−	−
A6	20	+	+	+	−	+	−	+ (strong)		+ (strong)	−

[a] AOAC method 29.107.

Commercial Glucose (Corn Sirup, Starch Sirup) in Honey

A new paper chromatographic procedure for detecting adulteration of honey with commercial glucose was described last year (2) and, on the basis of limited collaborative work, was accepted as first action by the Association (1). This year samples were sent to five collaborators outside this laboratory. Each collaborator received six unknown samples for testing and was requested to apply the new procedure and the older one, 29.107, which has been deleted by the Association (1). The only change in the procedure from that described last year is that two microliters are applied to the papergram instead of one microliter. Composition of the samples and the results of the tests are given in Table 3. Some comments of collaborators were as follows:

Collaborator No. 1.—"I used Mitchell's apparatus and technique (3). The time of development was 4 hours. The chromatograms had blue streaks without spots. This method is much better than the present AOAC method for the detection of low percentages of commercial glucose."

Collaborator No. 2.—"The chromatogram was prepared by ascending chromatography in the regular Mitchell developing tank . . . development time was 5½ hours. I believe the method to be clear, simple, and obviously more applicable than the present AOAC method."

Collaborator No. 3.—"I am very happy with the results I secured so far as the work permits me to interpret the chromatogram . . . the Mitchell tank with precut Whatman No. 1 was used . . . time of travel of solvent front was 3 hours. The paper chromatogram was removed to hood and left hanging overnight. New mobile phase was added next morning . . . again 3 hours for 6″ travel . . ." "The steps in handling the two precipitations with absolute alcohol were more indicative, to me, of the relative glucose amounts than was the old **29.107** procedure." For the latter, "the results were such that this analyst could only definitely state that known diluted Karo sirup contained commercial glucose. All others were so close to the same color as to not be clearly one or the other."

Collaborator No. 4.—"The streaks on samples 1 and 6 were considerably longer and stronger than the others. The AOAC test **29.107** did not give conclusive results—the colors were different only in degree . . . The test in my opinion leaves too much to the imagination or judgment and there is no indication where to draw the lines."

Color Classification of Honey

In response to a recommendation of Subcommittee D (1), a collaborative testing program was carried out on the USDA honey color classifier (4), the official USDA instrument for measuring honey color. F. L. Sutherland, Processed Products Standardization and Inspection Branch, Agricultural Marketing Service, designated five of their laboratories for the work. Six honey samples were sent to each of the laboratories to be classified by their USDA honey color classifier. Instructions, which are those routinely followed in the Branch, are as follows:

The clear blanks or the cloudy suspensions are placed in back of the glass standards in compartments 1, 3, and 5 of one or both of the comparators. The honey to be classified, which must be free of granulation, is poured into a clean dry bottle. The bottle is then placed in compartment 2 or 4 of either comparator box. The comparator is held at a convenient distance from the eye and viewed by diffused light (e. g., by north sky, overcast sky, or diffused artificial light source provided by a tungsten lamp or a white or daylight fluorescent lamp). The color classification of the honey is then determined by comparison of the sample with the standards. Switching the sample from compartment 2 to 4, or vice versa, interchanging the clear blanks and the appropriate cloudy suspension, and in some cases shifting to the second comparator or using both comparators, may be necessary.

If a sample is equal to the Water White standard in hue, or not as red (that is, yellower), the color is classified as Water White; if perceptibly redder than the Water White standard in hue, but not redder than the Extra White standard, the color is classified as Extra White; and so on. If redder in hue than the Amber standard, the color is classified as Dark Amber. It is emphasized that hue (amber quality or redness) is the attribute of color to be considered in this classification.

Most honeys are appreciably cloudy because of the presence of air bubbles and fine suspended matter. In such cases the brightness of a sample is lowered and its color classification may be difficult to determine, particularly if its hue is near that of one of the color standards. In such cases color classification will be more easily determined if the clear blank is replaced by one of the cloudy suspensions. These suspensions are intended only as aids in the classification for color and not intended as standards for "clarity," which is one of the factors scored in ascertaining the U.S. Grade of honey. They may in some cases, however, serve as aids in assessing clarity.

The results are given in Table 4. Agreement among laboratories is quite satisfactory.

Acidity of Honey

The Association has recommended (1)

Table 4. Collaborative study of honey color classification

Sample	Color[a]	Coll. 1	Coll. 2	Coll. 3	Coll. 4	Coll. 5
C1	Amber	A	A	A	A	A[b]
C2	Extra Light Amber	ELA	ELA	ELA	ELA	ELA
C3	Amber	A	A	A	A	A
C4	Light Amber	LA	LA	LA	LA	LA
C5	Extra Light Amber	[c]	ELA	ELA	ELA	ELA
C6	Amber	A	A	A	A	A

[a] As determined in Associate Referee's laboratory.
[b] Three other individuals in this collaborator's laboratory got identical results, with the exception of one classification of sample C3 as "light" amber.
[c] Sample lost in transit.

Table 5. Determination of acidity[a] of honey by two methods

Analyst	Proposed Method[b] Free	Lactone	Total	AOAC Method[c]
		Sample 246		
1	11.81	2.55	14.36	9.90
2	11.37	5.06	16.43	9.28
3	10.07	3.97	14.04	10.54
4	12.44	2.32	14.76	9.20
Av.	11.42	3.47	14.89	9.73
		Sample 266		
1	42.23	13.56	55.79	42.06
2	44.09	12.80	56.89	41.34
3	42.32	14.28	56.60	39.37
4	42.74	14.45	57.19	42.48
Av.	42.84	13.77	56.61	41.31
		Sample 351		
1	20.67	8.05	28.71	21.42
2	20.03	8.84	28.87	19.57
3	21.25	7.72	28.97	19.54
4	20.74	9.11	29.85	20.47
Av.	20.67	8.43	29.10	20.25
Standard Deviation	0.74	1.06	0.97	0.92

[a] Expressed as milliequivalents per kg honey.
[b] Each value is average of 3 determinations.
[c] Each value is average of 2 determinations.

that a recent procedure for determination of free, lactone, and total acidity of honey (5) be submitted to collaborative study.

In this procedure, a rapid titration of the free acidity is first made, giving values somewhat similar to those obtained by the official procedure (**29.106**). Excess alkali then is added, followed by immediate back-titration to pH 8.3. This eliminates the end-point drift frequently noted in the AOAC procedure and gives a value for lactone acidity, which is not determined in the official method.

As a preliminary study of the two procedures, three honey samples were titrated by both methods by four individuals in the laboratory of the Associate Referee. The results are given in Table 5. The standard deviations of the values obtained by the different analysts are of the same magnitude as that of the official method, though triplicates were run for the proposed method and duplicates for the AOAC method. Analyst 1 was experienced in the proposed method. The table shows that a substantial portion of the total acidity of honey is present in the lactone form, and the AOAC method does not measure this. Work done in the laboratory of the Associate Referee (6) has shown that gluconic acid (which equilibrates with gluconolactone) is the principal acid of honey.

Recommendations

It is recommended[1]—

(1) That collaborative work on the selective adsorption method for determination of honey sugars be continued.

(2) That the chromatographic procedure for detection of commercial glucose (corn sirup, starch sirup) in honey, now first action, be adopted as an official method.

(3) That the method for determining free, lactone, and total acidity previously

[1] These recommendations were approved by the General Referee and by Subcommittee D and were adopted by the Association. See *This Journal*, **43**, 138 (1960).

described in *This Journal,* **41**, 194 (1958) be submitted to further collaborative study.

(4) That the U.S. Department of Agriculture procedure for determination of color class of honey be adopted as first action.

(5) That the Schade method for determination of diastatic activity of honey be further studied collaboratively.

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